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Authors

Israel, E
Drazen, JM
Liggett, SB
et al.

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The Effect of Polymorphisms of the β_2-Adrenergic Receptor on the Response to Regular Use of Albuterol in Asthma

ELLIOT ISRAEL, JEFFREY M. DRAZEN, STEPHEN B. LIGGETT, HOMER A. BOUSHEY, REUBEN M. CHERNIACK, VERNON M. CHINCHILLI, DAVID M. COOPER, JOHN V. FAHY, JAMES E. FISH, JEAN G. FORD, MONICA KRAFT, SUSAN KUNSELMAN, STEPHEN C. LAZARUS, ROBERT F. LEMANSKE, Jr., RICHARD J. MARTIN, DIANE E. McLEAN, STEPHEN P. PETERS, EDWIN K. SILVERMAN, CHRISTINE A. SORKNESS, STANLEY J. SZEFLER, SCOTT T. WEISS, and CHANDRI N. YANDAVA for the National Heart, Lung, and Blood Institute's Asthma Clinical Research Network

Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; University of California at San Francisco, San Francisco, California; The Children's Hospital and Harvard Medical School, Boston, Massachusetts; Harlem Hospital Center, New York, New York; Montefiore Medical Center, New York, New York; Pennsylvania State University, Hershey, Pennsylvania; University of Wisconsin, Madison, Wisconsin; University of Cincinnati Medical Center, Cincinnati, Ohio; National Jewish Medical and Research Center, Denver, Colorado; and Thomas Jefferson University, Philadelphia, Pennsylvania

Inhaled β-adrenergic agonists are the most commonly used medications for the treatment of asthma although there is evidence that regular use may produce adverse effects in some patients. Polymorphisms of the β_2-adrenergic receptor (β_2-AR) can affect regulation of the receptor. Smaller studies examining the effects of such polymorphisms on the response to β-agonist therapy have produced inconsistent results. We examined whether polymorphisms at codon 16 (β_2-AR-16) and codon 27 (β_2-AR-27) of the β_2-AR might affect the response to regular versus as-needed use of albuterol by genotyping the 190 asthmatics who had participated in a trial examining the effects of regular versus as needed albuterol use. During the 16-wk treatment period there was a small decline in morning peak expiratory flow in patients homozygous for arginine at B_2-AR-16 (Arg/Arg) who used albuterol regularly. This effect was magnified during a 4-wk run out period, during which all patients returned to using as-needed albuterol, so that by the end of the study Arg Arg patients who had regularly used albuterol had a morning peak expiratory flow 30. 5 ± 12.1 L/min lower ($p = 0.012$) than Arg/Arg patients who had used albuterol on an as needed basis. There was no decline in peak flow with regular use of albuterol in patients who were homozygous for glycine at β_2-AR-16. Evening peak expiratory flow also declined in the Arg/Arg patients who used albuterol regularly but not in those who used albuterol on an as-needed basis. No significant differences in outcomes between regular and as-needed treatment were associated with polymorphisms at position 27 of the β_2-AR. No other differences in asthma outcomes that we investigated occurred in relation to these β_2-AR polymorphisms. Polymorphisms of the β_2-AR may influence airway responses to regular inhaled β-agonist treatment.

Inhaled selective β_2-agonists with an intermediate duration of action are the most commonly prescribed asthma medications in the world (1). Treatment of asthma by inhalation of agents such as albuterol, isoetharine, metaproterenol, pirbuterol, and terbutaline provides immediate and effective reversal of airway obstruction, with marked improvement in symptoms. Over the past several years, there has been considerable controversy about the role of inhaled β-agonists in the treatment of asthma (2–7). Specifically, it has been suggested that the regularly scheduled use of inhaled β-agonists is associated with a deleterious effect on asthma control.

We recently addressed this issue in patients with mild asthma by comparing, in a multicenter, placebo-controlled double-blind trial, asthma control in two cohorts, each of more than 125 patients (8). One cohort was treated with inhaled albuterol on a regularly scheduled basis, two puffs four times a day; the other was treated with an identical appearing inhaled placebo given on the same schedule. We found no clinically significant differences in overall asthma control between the two groups as a whole, despite the fact that the group allocated to regularly scheduled albuterol treatment used, on average, 7.2 puffs a day of inhaled albuterol whereas the as-needed only treatment group used only approximately 1¼ puffs per day. We concluded that, in patients with mild asthma, the regularly scheduled use of inhaled albuterol was not associated with either beneficial or deleterious effects.

While the above trial was in progress a number of polymorphisms of the β_2-adrenergic receptor (β_2-AR) were identified (9). Studies using mutagenesis and recombinant expression in cells (10, 11) and transgenic mice (12), and using airway smooth muscle cells endogenously expressing these β_2-AR variants (13), have shown that some forms of the β_2-AR display distinct differences in signaling and/or regulation after chronic exposure to β-agonists. It could thus be possible that these polymorphisms might explain altered pharmacologic responses to β-agonist treatment. In fact, recent studies have suggested that these polymorphisms may be associated with asthma of differing severity (14, 15). Further, other studies have reported a relationship between these polymorphisms and the degree of responsiveness or desensitization to the bronchodilator effect of β-agonists (16–19). However, these studies have produced inconsistent results. Altered desensitization to β-agonists has alternately been associated with either arginine or glycine polymorphisms at the 16 position of the β_2-AR and in other cases with polymorphisms at the 27 position. Many of these studies have been short-term, and several of these studies have compared asthmatics of differing severities in whom etiologic heterogeneity may influence apparent associations.

We therefore genotyped the subjects who participated in our earlier trial. We stratified the treatment cohorts and outcome measures with respect to genotype for the β_2-AR polymorphisms that occur most commonly in the population. Our data indicate that differences in β_2-AR genotypes are associated with altered responses to the regular use of albuterol.

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Correspondence and requests for reprints should be addressed to Elliot Israel, M.D., Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

METHODS

Inhaled β-Agonist Trial

The subjects in this report were participants in the National Heart, Lung, and Blood Institute (NHLBI) Asthma Clinical Research Network Trial on the effects of regular versus intermittent use of inhaled β-agonists (8). Two well-matched cohorts of patients with mild asthma ($FEV_1 \geq 70\%$ of predicted, provocative concentration of methacholine causing a 20% reduction in FEV_1 [PC_{20}] $\leq$ 8 mg/ml, and inhaled β-agonists as the only asthma treatment) were recruited at five centers across the United States. The patients were randomized to receive regular (two puffs 4 times a day) plus as-needed albuterol or as-needed albuterol alone, in a double-blind manner. The predetermined primary outcome variable for this study was morning (A.M.) peak expiratory flow. Additional monitoring included evening (P.M.) peak expiratory flow, peak expiratory flow variability, asthma symptom scores, the number of inhalations of rescue albuterol used, FEV_1, methacholine responsiveness, asthma-specific quality-of-life measures, and the acute response to inhaled albuterol. At the completion of the 16-wk randomized treatment period, all patients were switched in a single-blind fashion to regularly scheduled inhaled placebo for a 4-wk withdrawal period ("run out") in order to identify any deleterious effects of regularly scheduled albuterol treatment on lung function that may have been masked by the bronchodilation induced by the inhaled albuterol.

We found no differences in A.M. peak expiratory flow between the group treated regularly with albuterol and the group receiving intermittent albuterol, despite the fact that on average the regular treatment group used 7.2 puffs a day of albuterol whereas the as-needed group used only 1.3 puffs a day. There were no clinically significant differences between the groups in other physiologic or clinical variables monitored during the study. We concluded that, in patients with mild asthma, the regularly scheduled use of albuterol was not associated with either beneficial or adverse effects.

However, there were some patients who experienced a deterioration in peak expiratory flow during the study. At the end of the trial, we contacted all participants who had been randomized and collected either blood or buccal brushings to obtain cellular material for genotyping. Patients who could not return to their clinical center were mailed cheek brushes to use and to return to the laboratory by mail. Additional informed consent for genotyping was obtained from all participants at all study sites. Material for genotyping was obtained from 190 of 255 randomized patients.

Genotypic Analysis

Terminology. Two alleles have been identified for each of the common polymorphisms at amino acids 16 and 27 (20). At amino acid 16 of the β_2-AR, the alternative alleles contain either glycine (Gly) or arginine (Arg). The three possible genotypes at this locus are termed B16-Arg/Arg, B16-Arg/Gly, or B16-Gly/Gly. At amino acid 27, the alternative alleles contain either glutamic acid (Glu) or glutamine (Gln). The three possible genotypes at this locus are termed B27-Gln/Gln, B27-Gln/Glu, or B27-Glu/Glu.

Assessment of genotype. Genotyping was performed by individuals who were unaware of the results from the clinical trial. Genomic DNA was prepared for genotypic analysis by standard techniques (21). Genotypes at the B16 and B27 position were assessed by the amplification refractory mutation system (ARMS) (22, 23) similar to that previously described (14). Genotype was assigned in approximately 10% of individuals by oligonucleotide-specific hybridization as a quality control measure throughout the study.

Statistical Analysis

The statistical analysis is similar to that described for the Asthma Clinical Research Network (ACRN) β-agonist trial (8). Briefly, because of the longitudinal nature of most of the response variables, a mixed-effects linear model was applied (24, 25); this approach allowed the use of all data obtained, not just the data obtained at a single visit. This statistical model was determined before the start of the study, and therefore other models were not considered during data analysis. A Bonferroni correction was applied for the three pairwise comparisons among genotypes, thereby reducing the significance level to 0.0167.

TABLE 1

DISTRIBUTION, BY TREATMENT GROUP, OF SUBJECTS FOR WHOM GENOTYPIC INFORMATION WAS OBTAINED

	In the Treatment Trial	Genotype Data Available	
		B16	B27
Regularly scheduled Rx	126	96	96
As-needed Rx	129	83	81

Definition of abbreviation: Rx = treatment.

RESULTS

Genotypes

Material for genotyping was obtained from 190 of the 255 subjects in the trial. At the B16 and B27 loci, a definite genotype could be assigned in 179 and 177 individuals, respectively. The distribution of patients for whom genotypic information was obtained is shown in Table 1. The allele frequency of B16-Arg and Gly was 0.4 and 0.6, respectively, and of B27-Gln and Glu 0.6 and 0.4, respectively. The number of individuals possessing each of the potential genotypes at each locus individually (B16 or B27) was consistent with the Hardy-Weinberg equilibrium. A total of 173 individuals were successfully genotyped at both loci. The distribution of the various combinations of heterozygous and homozygous polymorphisms at positions 16 and 27 is shown in Table 2. It is interesting that all individuals with the B16-Arg/Arg genotype had the B27-Gln/Gln genotype.

Results Stratified by Genotype

There were no significant differences in the baseline characteristics when we stratified our subjects by genotype (Table 3). We examined the effects of regular versus as-needed albuterol use over the 20 wk from the time of randomization through the end of the run-out, stratified by genotype (*see* METHODS). In B16-Arg/Arg patients, but not in any of the patients with alternate genotypes, regular β-agonist use was associated with a decline in the primary outcome indicator—A.M. peak expiratory flow, and a decline in the secondary outcome indicator—P.M. peak expiratory flow (Table 4). These changes did not occur in any of the other B16 genotypes or any of the B27 genotypes (Table 4). In B16-Arg/Arg patients, regular β-agonist treatment produced a fall in A.M. peak expiratory flow whereas as-needed treatment produced a slight rise in peak expiratory flow (Figure 1). In these patients, the difference in the change

TABLE 2

NUMBER OF SUBJECTS WITH EACH OF THE POTENTIAL GENOTYPE COMBINATIONS

Genotype		No. of Subjects	Treatment Group	
B16	B27	Observed	Regular	As-needed
Arg/Arg	Gln/Gln	26	16	10
Arg/Gly	Gln/Gln	29	15	14
Gly/Gly	Gln/Gln	7	3	4
Arg/Arg	Gln/Glu	0	0	0
Arg/Gly	Gln/Glu	58	29	29
Gly/Gly	Gln/Glu	27	15	12
Arg/Arg	Glu/Glu	0	0	0
Arg/Gly	Glu/Glu	0	0	0
Gly/Gly	Glu/Glu	26	16	10
Total		173	94	79

Definition of abbreviations: Arg = arginine; Gln = glutamine; Glu = glutamic acid; Gly = glycine.

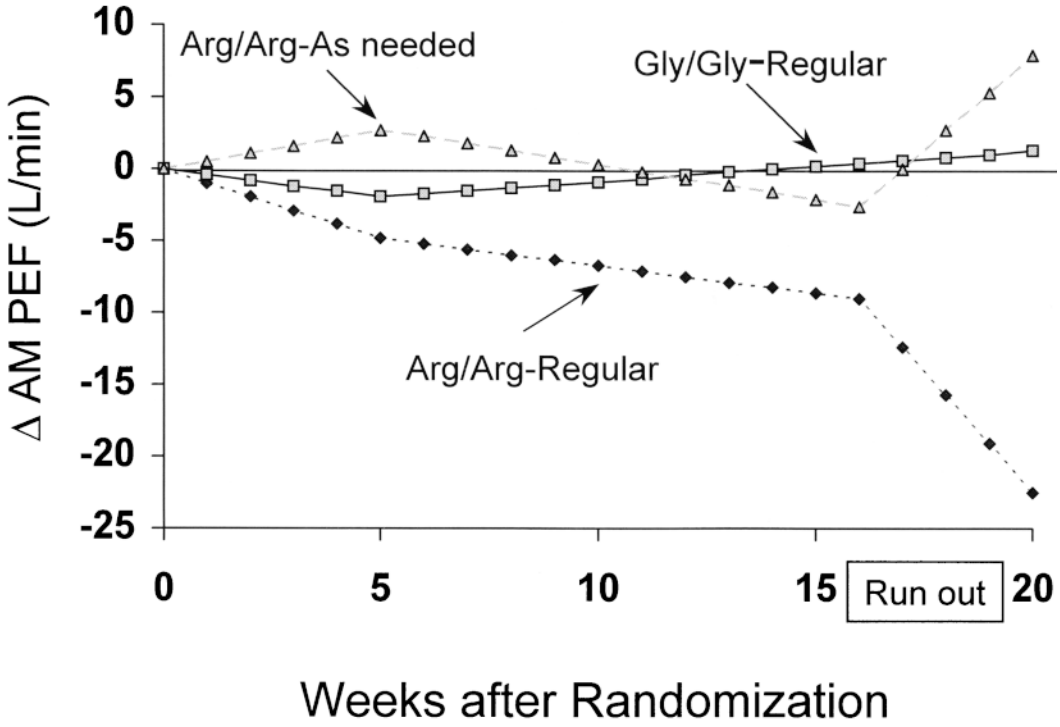

Figure 1. Time course of the change in morning peak expiratory flow (A.M. PEF) among different B16 genotypes in response to β-agonist treatment. Over the treatment and run-out period, B16-Arg/Arg patients who received regularly scheduled β-agonist treatment (Arg/Arg-Regular) experienced a 30.5 ± 12.1 L/min decline in A.M. peak expiratory flow relative to those who received as-needed treatment (Arg/Arg-As needed) ($p = 0.012$). B16-Gly/Gly patients were not affected by regular treatment (Gly/Gly-Regular). Thus, regular treatment was associated with a 23.8 ± 9.5 L/min decline in peak expiratory flow in B16-Arg/Arg patients relative to B16-Gly/Gly ($p = 0.012$). Values were derived from the statistical analysis model described in METHODS. Run-out = predetermined 4-wk period when regular β-agonist use had been discontinued.

in peak expiratory flow between regularly scheduled and as-needed treatment over the study period was 30.5 ± 12.1 L/min ($p = 0.012$, Figure 1, Table 4). The decline in peak expiratory flow produced by regularly scheduled β-agonist treatment was restricted to the B16-Arg/Arg patients. B16-Gly/Gly patients who received regularly scheduled treatment had no drop in peak expiratory flow (Figure 1, Table 4). Their A.M. peak expiratory flow was 23.8 ± 9.5 L/min greater than that of the B16-Arg/Arg patients who received regularly scheduled treatment ($p = 0.012$, Figure 1).

The patterns of change were similar for the secondary outcome indicator, P.M. peak expiratory flow (Figure 2, Table 4). The P.M. peak expiratory flows of B16-Arg/Arg subjects who received regular treatment fell 31.1 ± 13.0 L/min compared with those B16-Arg/Arg patients who received as-needed treatment only ($p = 0.0167$). Once again, the effect of regular treatment occurred only in those with the B16-Arg/Arg genotype. B16-Gly/Gly patients who received regular β-agonist treatment did not experience a drop in mean P.M. peak expiratory flow, and their P.M. peak expiratory flow was 31.6 ± 10.2

TABLE 3

BASELINE CHARACTERISTICS OF SUBJECTS BY GENOTYPE*

	B16			B27		
Characteristic	Arg/Arg (*n* = 28)	Arg/Gly (*n* = 89)	Gly/Gly (*n* = 62)	Gln/Gln (*n* = 62)	Gln/Glu (*n* = 87)	Glu/Glu (*n* = 28)
Male sex, n (%)	11 (39.3)	45 (50.6)	20 (32.3)	24 (38.7)	43 (49.4)	8 (28.6)
Minority group, n (%)	10 (35.7)	25 (28.1)	18 (29.0)	27 (43.6)	21 (24.1)	5 (17.9)
Atopy, n (%)	25 (89.3)	89 (100.0)	59 (95.2)	58 (93.6)	87 (100.0)	26 (92.9)
Age, yr	30.4 ± 10.1	27.7 ± 9.1	29.9 ± 9.7	29.3 ± 9.9	28.4 ± 9.5	29.5 ± 8.0
Age < 18 yr, n (%)	3 (10.7)	14 (15.7)	7 (11.3)	8 (12.9)	13 (14.9)	2 (7.1)
A.M. peak flow, L/min†	389.1 ± 84.7	427.7 ± 100.2	395.3 ± 95.3	406.9 ± 92.9	419.6 ± 102.8	389.5 ± 91.0
P.M. peak flow, L/min†	417.4 ± 90.7	444.8 ± 105.1	418.2 ± 91.6	424.9 ± 91.5	441.9 ± 107.7	416.6 ± 87.4
Peak flow variability, %†‡	5.1 ± 10.1	3.0 ± 7.3	4.3 ± 9.3	3.4 ± 8.4	4.0 ± 8.4	4.9 ± 8.5
Symptom score†§	0.35 ± 0.38	0.39 ± 0.37	0.49 ± 0.45	0.39 ± 0.40	0.42 ± 0.42	0.48 ± 0.36
Rescue β-agonist use†	1.2 ± 2.0	1.5 ± 2.4	1.5 ± 1.9	1.6 ± 2.3	1.5 ± 2.2	1.4 ± 1.2
FEV_1, L	2.92 ± 0.73	3.24 ± 0.76	3.02 ± 0.70	2.97 ± 0.74	3.25 ± 0.78	3.02 ± 0.56
FEV1, % pred‖	88.5 ± 12.8	90.0 ± 12.6	90.0 ± 14.0	90.2 ± 12.1	89.3 ± 14.2	89.4 ± 10.5
Quality-of-life score‖¶	2.19 ± 0.88	2.25 ± 0.74	2.41 ± 0.92	2.25 ± 0.80	2.36 ± 0.83	2.34 ± 0.91
PC_{20}, mg/ml‖**	0.80 (0.38, 2.14)	0.90 (0.31, 2.10)	0.74 (0.24, 3.00)	1.14 (0.43, 3.17)	0.70 (0.25, 1.82)	0.82 (0.28, 3.51)
Reversibility††	11.3 ± 10.4	9.4 ± 11.4	10.6 ± 8.8	8.6 ± 8.5	11.4 ± 12.1	10.2 ± 9.2

* Values are means ± SD unless otherwise indicated.

† Values represent averages for the sixth (final) week of the run-in period.

‡ Peak flow variability was calculated as ([evening peak flow − morning peak flow] ÷ evening peak flow) × 100.

§ Asthma symptoms were graded by the patient each day, from 0 for no symptoms to 3 for incapacitating symptoms.

‖ This characteristic was measured from Week 6 of the run-in period.

¶ Asthma-specific quality-of-life questionnaires were completed by the patients during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; a score of 2.0, that the patient's life was "a little limited" by asthma; a score of 3.0, that there was "some limitation"; and a score of 7.0, that there was "total limitation."

** Geometric mean (Interquartile range).

†† Percentage change in FEV_1 from baseline in response to albuterol inhalation. Data are from Week 4 of the run-in period.

TABLE 4

DIFFERENCE BETWEEN THE EFFECT OF REGULAR AND AS-NEEDED β-AGONIST USE COMPARING END OF WITHDRAWAL TO RANDOMIZATION STRATIFIED BY GENOTYPE AT THE B16 AND B27 LOCI*

	B16			B27		
	Arg/Arg	Arg/Gly	Gly/Gly	Gln/Gln	Gln/Glu	Glu/Glu
A.M. Peak expiratory flow, L/min	−30.5 ± 12.1†	2.6 ± 6.6	−8.8 ± 8.0	−14.3 ± 8.1	−3.1 ± 6.8	10.5 ± 12.3
p Value	0.0123	0.699	0.268	0.076	0.645	0.393
CI‡	(−59.8, −1.1)	(13.5, 18.6)	(−28.1, 10.4)	(−33.9, 5.2)	(−19.6, 13.3)	(−19.3, 40.4)
P.M. Peak expiratory flow, L/min	−31.1 ± 13.0†	−5.7 ± 7.1	−4.1 ± 8.5	−14.1 ± 8.7	−10.4 ± 7.3	12.9 ± 13.2
p Value	0.0167	0.428	0.634	0.104	0.152	0.327
CI‡	(−62.6, 0.4)	(−22.9, 11.6)	(24.8, 16.6)	(−35.0, 6.9)	(−28.0, 7.2)	(−19.0, 44.8)
A.M. Peak expiratory flow, % pred	−7.00 ± 2.63	0.32 ± 1.48	−1.90 ± 1.76	−3.19 ± 1.76	−0.96 ± 1.52	2.24 ± 2.67
p Value	0.008	0.828	0.281	0.070	0.527	0.402
CI‡	(−13.37, −0.62)	(−3.25, 3.90)	(−6.15, 2.36)	(−7.45, 1.08)	(−3.93, 3.31)	(−4.22, 8.69)
P.M. Peak expiratory flow, % pred	−6.93 ± 2.74	−1.55 ± 1.53	−0.79 ± 1.83	−3.29 ± 1.82	−2.44 ± 1.57	2.60 ± 2.76
p Value	0.011	0.312	0.667	0.071	0.120	0.346
CI‡	(−13.55, −0.31)	(−5.27, 2.16)	(−5.21, 3.64)	(−7.70, 1.12)	(−6.23, 1.35)	(−4.07, 9.23)
FEV_1 (L)	0.14 ± 0.11	0.01 ± 0.06	−0.10 ± 0.07	0.09 ± 0.07	−0.02 ± 0.06	−0.09 ± 0.12
p Value	0.190	0.940	0.189	0.265	0.802	0.453
CI‡	(−0.12, 0.41)	(−0.14, 0.15)	(−0.27, 0.08)	(−0.10, 0.28)	(−0.18, 0.15)	(−0.38, 0.20)
PC_{20}§	1.21 ± 0.61	−0.25 ± 0.34	−0.63 ± 0.41	0.20 ± 0.40	−0.30 ± 0.34	−0.69 ± 0.64
p Value	0.047	0.459	0.126	0.621	0.380	0.279
CI‡	(−0.26, 2.67)	(−1.07, 0.57)	(−1.62, 0.36)	(−0.77, 1.17)	(−1.12, 0.52)	(−2.24, 0.85)
Peak expiratory flow variability, %	−1.2 ± 2.2	−1.8 ± 1.2	1.9 ± 1.4	−0.5 ± 1.5	−1.0 ± 1.2	0.5 ± 2.2
p Value	0.577	0.126	0.184	0.744	0.418	0.834
CI‡	(−6.4, 4.0)	(−4.7, 1.0)	(−1.5, 5.3)	(−4.0, 3.1)	(−3.9, 2.0)	(4.9, 5.9)

* Values ± SEM represent the effect of as-needed use subtracted from effect of regular use where the "effect" represents the change between the end of run-out and the start of randomization. Negative values indicate a decline associated with regular use.
† Value at or below threshold of significance of 0.0167 as outlined in text.
‡ 98.33% confidence interval (adjusted for modified p value).
§ Doubling dose change.

L/min greater than the B16-Arg/Arg patients who received regular treatment (p = 0.0019, Figure 2). The decrease in A.M. and P.M. peak expiratory flow in response to regular β-agonist use held true even when the B16 heterozygotes were included with the Gly/Gly homozygotes. When the B16-Arg/Arg subjects were compared with all B16-non-Arg/Arg subjects as a group (B16-Arg/Gly and B16-Gly/Gly), the A.M. peak expiratory flow difference was 26.6 ± 8.6 (p = 0.0019) L/min and the P.M. peak expiratory flow difference 30.6 ± 9.2 L/min (p = 0.0009) (data not shown). The A.M. and P.M. peak expiratory flow differences also held true when these differences were expressed as a percent of predicted peak expiratory flow and represented a 7% difference owing to regular β-agonist use (Table 4).

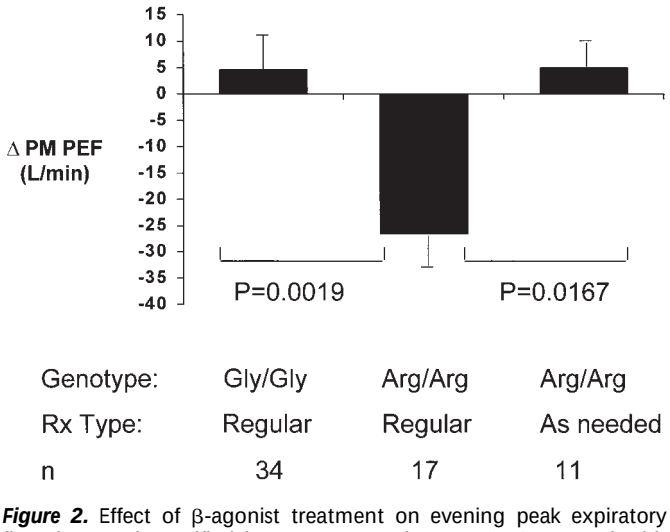

Figure 2. Effect of β-agonist treatment on evening peak expiratory flow (P.M. PEF) stratified by genotype at locus B16. Compared with peak expiratory flow at randomization, at the end of 20 wk, B16-Arg/Arg patients who received regularly scheduled β-agonists (Regular) experienced a decline in P.M. peak expiratory flow compared with those who received as-needed treatment (p = 0.0167). Regularly scheduled treatment did not produce a decline in P.M. PEF in B16-Gly/Gly patients. The difference in the change in P.M. PEF between B16-Gly/Gly and B16-Arg/Arg patients who received regularly scheduled treatment was 31.6 ± 10.2 L/min (p = 0.0019).

There were no clinically significant B16 genotype–related differences in any of the other secondary outcome indices monitored. With respect to the B27 locus, there were no significant differences between individuals harboring each of the genotypes in any of the outcomes monitored. There were also no differences in asthma exacerbations and treatment failures among genotypes by treatment (Fisher exact test).

DISCUSSION

In this report, using a large cohort of well characterized, mild asthmatics (190 patients), we demonstrate that regular use of β-agonists can produce distinct effects on airway function in patients with specific polymorphisms of the β-adrenergic receptor. Regular use, as opposed to as-needed use, reduced both A.M. and P.M. peak expiratory flow in patients homozygous for the Arg-16 allele. This deterioration in pulmonary function associated with regular albuterol use was particular to the Arg-16 allele because patients homozygous for the Gly-16 allele did not experience such an effect. Further, we found that polymorphisms at amino acid 27 of the β-adrenergic receptor did not alter the response to regular β-agonist use in these asthmatic patients.

In vitro studies have demonstrated that B16-Arg and B16-Gly variants of the β-adrenergic receptor do not differ in terms of receptor binding characteristics or receptor-mediated activation of the adenyl cyclase second messenger pathway. However, they do

differ in the extent to which the respective receptors are downregulated in response to long-term catecholamine exposure (10–13). Thus, β_2-AR polymorphisms might alter the response to the use of β-agonists. However, the clinical effects and associations noted with β_2-AR polymorphisms have been contradictory in nature. Several of the associations have been derived from studies that contained small groups of patients and/or asthmatics who were heterogeneous in terms of disease severity.

We were able to genotype (in a blinded manner) 190 carefully defined patients available from our study of regular versus as-needed β-agonist use and to examine the influence of genotype at the β_2-AR on the effect of regular use of albuterol on our predetermined primary outcome variable, A.M. peak expiratory flow. Peak expiratory flow had been chosen as the primary outcome variable for the prior study because it is a well-documented indicator of deteriorating asthma control (26, 27). It is a measurement that was obtained daily from our patients, thus providing a large number of data points for each patient. We performed our primary comparison in homozygous individuals because we believed that heterozygous individuals might have an intermediate phenotype that might have been difficult to define.

We found that regular albuterol use was associated with a decline in A.M. and P.M. peak expiratory flow in patients who are B16-Arg/Arg. These data suggest that patients with the B16-Arg/Arg polymorphism may be at risk for adverse effects, or less of a salutary effect, when using β-agonists regularly. This is of particular importance because many patients with mild asthma will increase the frequency of β-agonist use during asthma exacerbations. Our data suggest that a proportion of these patients (the approximately 15% of patients who are Arg/Arg at B16), may not benefit to the same degree as the general population, when they use their β-agonists regularly and may actually experience a decline in airway function, especially as they discontinue high-dose β-agonist therapy. Whether concomitant inhaled corticosteroids would blunt this adverse effect is unclear. However, more than 70% of patients with asthma use β-agonists as their only form of therapy and will increase β-agonist use with exacerbations.

The A.M. peak expiratory flow difference that occurred in the B16-Arg/Arg patients was greater than 30 L/min. A decline of this magnitude has been associated with significant clinical deteriorations in asthmatics. For example, declines of 19 and 23 L/min in A.M. and P.M. peak expiratory flow, respectively, have been reported in patients taken off inhaled corticosteroids and were associated with clinical deteriorations (28). A 25 L/min difference occurred between asthmatics treated with regular inhaled corticosteroids versus those treated with regular β-agonists in a major study by Haahtela and colleagues (29). It is therefore of interest that we did not observe differences, that varied by genotype, in our secondary outcome variables such as peak flow variability or PC_{20}.

Previously published studies have suggested that other β-agonist genotypes may be associated with asthma of differing severity or other markers associated with asthma. Patients with nocturnal asthma were more likely to have the B16-Gly form of the receptor (14). B27-Gln has been associated with elevated levels of IgE (30). In another study, B27-Glu has been associated with a lower degree of airway reactivity than B27-Gln (15). In contrast to our study, these studies encompassed a wide range of asthmatics, including moderate to severe asthmatics. Because our patients were all chosen to be mild asthmatics, we did not expect to have a wide enough range of asthmatics to detect relationships related to severity. For instance, in our population, the peak mean peak expiratory flow difference was approximately 7% of the baseline peak expiratory flow and thus may not have been adequate to precipitate appreciable functional changes in this mild population over such a short time period. However, in a more severe population of asthmatics such a decline, if it occurred, might have more profound and more rapid clinical implications. It is thus of interest, that in the slightly more severe population of asthmatics reviewed previously, a worsening of airway reactivity did in fact occur in the B16-Arg homozygotes (18).

It also worth noting that the majority of the decline in peak expiratory flow in the B16-Arg/Arg patients occurred in the run-out, after patients had stopped using their albuterol regularly. We had specifically designed the run-out period of this study because of a concern that the bronchodilating effect of the regular β-agonist use might mask a deleterious effect. The precise mechanism of this postalbuterol deterioration in disease control is unclear. Although rebound effects occur after withdrawal of β-agonists, it is not clear that they are long-lived enough to explain the effect we observed.

Although our study was not designed to explain the mechanism of the decline in airway function that occurred only in the B16-Arg/Arg subjects who used regular albuterol, our knowledge of the properties of the alternate forms of the receptors may explain our findings. B16-Gly expression downregulates to a greater extent than B16-Arg after exposure to catecholamines (11). Taken alone, these data would suggest that tachyphylaxis to the effect of regular exogenous β-agonists would occur to a greater degree with B16-Gly. However, in a proposal of a so-called "dynamic model" of receptor kinetics (20), it has been suggested that endogenous catecholamines actively downregulate the β_2-AR at baseline. Thus, in the resting state, Gly16 (the variant more susceptible to downregulation) would be downregulated to a greater extent than Arg16 by endogenous catecholamines. It then follows that the tachyphylactic effect of regular exogenous exposure to β-agonists would be most apparent in Arg16 patients because their receptors have not yet been downregulated. Further, this dynamic model would predict that the initial response to albuterol would be depressed in individuals with the Gly16 polymorphism, because their receptors have been endogenously downregulated to a greater extent than in patients with the Arg16 polymorphism. The findings of Martinez and coworkers (19) are in concert with this model because they found that B16-Arg/Arg patients have an enhanced bronchodilator response to albuterol. In contrast, reports in two much smaller studies have found decreased responses, or greater degrees of tachyphylaxis, associated with Gly16 or Gln27 (16, 17). However, the latter study involved the β-agonist formoterol, which has unique interactions with the β-receptor. Our findings of a lack of effect of genotypic variants at the B-27 locus are also consistent with the *in vitro* studies. Whereas B27-Gln has a greater tendency to downregulation than B27-Glu, these effects are overcome by the downregulation phenotype at B16.

Although the effects we observed are consistent with the effects predicted by the dynamic model for the Arg/Arg genotype at position 16, we cannot rule out the possibility that the mechanism of this effect may be totally unrelated to the downregulation of the receptors. Rather, it is possible that the B16-Arg genotype is in linkage disequilibrium with a polymorphism nearby on the genome. For example, the Arg16 polymorphism has recently been shown to be in linkage disequilibrium with a polymorphism at the 5′ leader cistron, which is 102 base pairs upstream of the β_2-AR coding block and codes for a peptide that influences the translation of the β_2-AR gene (31). While this specific polymorphism is also in linkage disequilibrium with Gln27 as well, making it unlikely to be the source of the association we observed, other polymorphisms may yet be identified. In this regard, there is a linkage between B16-Arg and B27-Gln so that haplotypically all patients who are B16-Arg possess B27-Gln (*see* Table 2). An analysis of the sub-

group of patients who were B27-Gln/Gln showed that the adverse effect of regular use of β-agonists was still attributable to the Arg/Arg genotype (data not shown). Regardless of the mechanism of the effect, the association we observed suggests that the Arg16 polymorphism, at the very least, clinically serves as a marker for an altered pharmacologic response to β-agonists.

In summary, we have demonstrated that the homozygous arginine genotype at position 16 of the β_2-AR can influence the response to use of a β-agonist. The altered response in these patients occurs only with regular use, as compared with as-needed use. Most asthmatics, whether using concomitant anti-inflammatory therapy or not, increase their β-agonist use during exacerbations. Approximately 15% of the population is homozygous for Arg 16. If corroborated, our findings suggest that these individuals may benefit by avoiding regularly scheduled β-agonists and might be candidates for earlier intervention with anti-inflammatory agents.

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APPENDIX

Additional Asthma Clinical Research Network Investigators: J. D. Spahn, National Jewish Medical and Research Center, Denver, CO; T. J. Craig, and E. A. Mauger, Milton S. Hershey Medical Center, Hershey, PA; S. A. Nachman, The Harlem Hospital Center, New York, NY; C. V. Chambers, K. R. Epstein, and S. J. McGeady, Thomas Jefferson University, Philadelphia, PA